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Association of *Fat Mass and Obesity Associated*, *Dopamine Receptor Type 2* and *Ankyrin Repeat and Kinase Domain Containing 1* Genes with Pediatric Obesity and Metabolic Risk: A Case-Control Study

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ABSTRACT

Background
Genetic polymorphisms that affect the availability and secretion of dopamine can affect the risk of obesity.

Objectives
To investigate the relationship between pediatric obesity and cardiovascular risk factors (CRF) with the polymorphisms of "*Fat Mass and Obesity Associated*" (*FTO*) rs9939609, "*Dopamine Receptor type 2*" (*DRD2*) rs6277 and "*Ankyrin Repeat and Kinase Domain Containing 1*" (*ANKK1*) rs18000497 genes.

Methods
Case-Control study conducted with 226 pediatric patients from 5 to 16-years of age. The two main groups, Obese (O) and Eutrophic (E), were subdivided according to the value of HOMA-IR into obese with insulin resistance (ORI) or insulin sensitivity (OSI) and eutrophic resistant (ERI) or sensitive (ESI) to insulin. According to the presence of two or more *CRF*, they were subdivided into metabolically unhealthy or metabolically healthy groups: Obese Metabolically Unhealthy (OMU), Obese Metabolically Healthy (OMH), Eutrophic Metabolically Unhealthy (EMU) and Eutrophic Metabolically Healthy (EMH). Polymorphisms were determined by real-time Polymerase Chain Reaction (PCR) or Restriction Fragment Length Polymorphisms (PCR-RFLP).

Results
In the obese group, the higher the number of risk alleles of *FTO* and *ANKK1* genes isolated and the three genes combined, the higher the mean BMI (p<0.0001). Regarding the *FTO* gene: the frequency of the risk allele was: 57.7%-ERI, 37.4%-ESI (p=0.048), and the homozygous wild genotype was: 29.5%-OMU, 37.5%-OMH (p=0.02). Regarding the *DRD2* gene: the genotypes with the risk allele were present in 84.6%-OMU and 67.5%-OMH (p=0.031). Regarding the *ANKK1* gene: the frequency of the homozygous risk genotype was current in 15.4%-ERI and 13.5%-ESI (p<0.0001) and 62.5%-EMU and 41.5%-OMH (p=0.031).

Conclusion
Risk alleles of *FTO*, *DRD2* and *ANKK1* genes had an additive effect on the outcome of pediatric obesity in Brazilian children and conferred a higher risk of insulin resistance (*FTO* and *ANKK1*) and CRF.

Keywords
Childhood obesity; Genetic polymorphism; Insulin resistance; Metabolic syndrome; Dopamine.

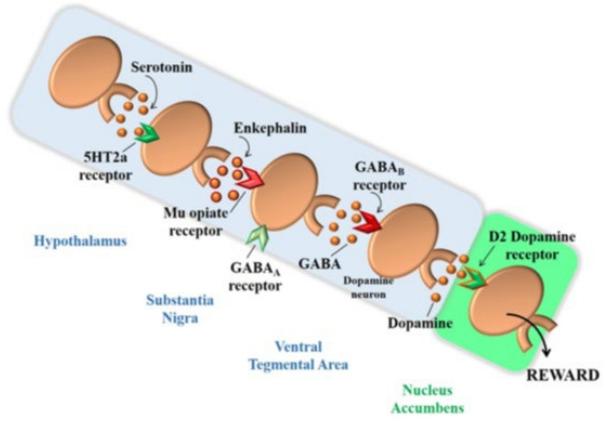
INTRODUCTION

Obesity is recognized by the World Health Organization (WHO) as one of the ten leading health problems in most diverse societies,[1] and in 2019 chronic non-communicable diseases: obesity, diabetes, cancer, and cardiovascular diseases reached the second position in the ranking world of health challenges.[2] The prevalence of obesity has increased worldwide, with epidemic proportions in many developed and developing countries.[3,4] Currently, obesity is considered a neurobehavioral disease in which hypothalamic changes occur in the control of hunger, satiety, and energy expenditure.[5] It is a condition of multifactorial etiology influenced by genetic, epigenetic, endocrine-metabolic, and behavioral factors.[6,7]

The regulation of food intake by the Central Nervous System (CNS) depends on the interaction of a homeostatic component that aims to balance energy and nutrients and a hedonic component, which seeks pleasure associated with food.[8,9] The ingestion of highly palatable foods (rich in sugar and fats) can deregulate the homeostatic control of appetite, perpetuating the stimulus to eat, which makes the intake primarily mediated by hedonic and non-homeostatic needs.[10]

Cortico-limbic-striated structures and circuits form the Brain Reward System (BRS). BRS is activated by various stimuli that trigger a cascade of neurotransmitter secretion that ultimately releases dopamine (DA), bringing a sense of pleasure and leading the individual to seek positive reinforcements.[10,11] The brain reward cascade starts in the hypothalamus, where serotonin acts as a neurotransmitter stimulating the enkephalin release, which in turn inhibits GABAergic neurons in the substantia nigra. These GABAergic neurons act in the fine adjustment of DA amount that will be released in the NAc, the brain reward site (Figure 1).[12]

Figure 1. *Interaction of Various Neurotransmitters that forms the "Brain Reward Cascade*



Source: Pinto et al[12]

Considering that DA plays a crucial role in BRS, it is involved in eating behavior, and that genetic factors are responsible for up to 80% of the inter-individual variation of nutritional status,[13] the study of genetic polymorphisms that affect the availability and secretion of DA have gained space in the literature.[12]

Dopamine Receptor Type 2 gene (rs6277) Polymorphism

The *dopamine receptor type 2* (*DRD2*) is the main self-regulator of DA release in the brain reward dopaminergic system, with pre and postsynaptic effects.[14]

The *DRD2* gene, located at 11q22-q23, has several polymorphisms capable of influencing the quantity and functionality of receptors in the membrane of the postsynaptic cell, which directly implies in the predisposition of individuals to seek any sub stance or behavior that stimulates BRS[15] with a consequent higher propensity to gain weight in the future.[16]

The single nucleotide polymorphism (SNP) *rs6277* of the *DRD2* gene consists of the change of an Arginine (A) by Guanine (G) in the position 957pb in exon 7. There are only a few researches that studied this polymorphism from a metabolic point of view, but it is known that the presence of the risk allele (G) leads to a reduction in the affinity of the receptors and is associated with: psychiatric disorders related to the deficiency of dopaminergic function and processing of emotions,[17] a greater sense of reward even in individuals of normal weight,[18] higher consumption of sugar and increased body mass index (BMI) from normal to overweight/obesity in adults.[19]

Ankyrin Repeat and Kinase Domain Containing 1 gene (rs1800497) Polymorphism

The *Ankyrin repeat and Kinase domain containing 1* gene (*ANKK1*) is also located in the 11q22-q23 region. It's *rs1800497* SNP interferes with the availability of *DRD2* receptors and was considered part of the *DRD2* gene itself, under the name of Taq1A polymorphism of the *DRD2* gene.[20] This SNP influences the signaling of *DRD2* receptors: healthy individuals who have the risk allele (T) have a reduction of between 30-40% in the density of *DRD2* in the striatum when compared to those without T-allele, and reduced glucose metabolism in the regions of BRS that contain dopaminergic neurons.[20] This reduction in the BRS's dopaminergic action reflects clinically in a higher risk of obesity in adults,[16] greater weight gain,[16] more intense reinforcement (feeling of pleasure) after a meal, and increased energy intake, especially in obese.[21] There is a wide variation in genotype and allele frequencies in different countries, and even within the same country, there is inconsistency regarding the association or not with metabolic outcomes. Chart 1 shows the main results of these scientific researches.

Fat Mass and Obesity Associated gene (rs9939609) Polymorphism

The *Fat Mass and Obesity Associated* (*FTO*) gene, located at 16q12.2, encodes a 2-oxoglutarate-dependent demethylase involved in post-translational modifications, deoxyribonucleic acid (DNA) repair, and fatty acid acidification.[22] The presence of at least one risk allele (A) of this SNP is associated with higher caloric intake, less satiety, and a higher frequency of episodes of compulsive eating when compared with homozygotes for the wild allele (T).[23-26]

Chart 1. *Summary of Studies Carried Out in the Pediatric Population that Evaluated the rs1800497 Polymorphism of the ANKK1 gene*

Reference	Population	Conclusion
Epstein et al[84]	26 Children from 8 to 12-years old with overweight and parents with obesity Country: USA	Presence of the T allele associated with higher BMI and also weight modification after a weight loss program lasting 0.5 to 1-year.
Ergum et al[85]	Children 46 obese 50 eutrophic Country: Turkey	There was no difference in the allelic distribution of the groups. The following proportion was observed: Obese: T- 51%, C- 49% Eutrophic: T- 52% C- 48%
Stice et al[16]	44 female teenagers Country: USA	Observation of MRI in a situation of food anticipation showed attenuated response of the CNS of patients with the presence of the T allele.
Strien et al[86]	279 Adolescents Country: Netherlands	Genotype frequency: TT-2.9%; CT - 30.8%; CC - 66.3% Allele frequency: T -18.3%; C- 81.7% The presence of the T allele increased the intake of emotional background
Duran-Gonzalez et al[87]	448 Adolescents Country: USA	Genotype and allele frequency (%): TT-15.2; CT- 46.5; CC- 38.3; T-38.5; C- 61.5 T allele associated with central obesity
Araz et al[42]	200 pediatric patients aged 2 to 17 years. 50% obese 50% eutrophic Country: Turkey	Genotypic and allele frequency (%) in Obese: TT-7; CT-26; CC- 67 T-20; C - 80 Genotype and allele frequency (%) in Controls: TT-4; CT-31; CC- 65 No association of T allele and BMI
Roth et al[88]	583 eutrophic adults 28 overweight children 423 children with obesity Country: USA	n children, the following genotypic and allelic frequencies were found: TT-2.4%; CT- 29.3%; CC- 68.3% T- 17%; C- 83% There was no association between elevated BMI with the presence of the T allele; however, the TT genotype showed a worse response after a lifestyle change program aimed at weight loss.
Pinto et al[76]	Pediatric patients from 5 to 16 years of age 55 obese 50 eutrophic Country Brazil	Genotype and allele frequency (%) in the obese: TT-14.5; CT-40; CC-45.5 T-34.5; C 65.5 Genotype and allele frequency (%) in controls: TT-10; CT-26; CC-64 T-23 allele; C-77 T allele associated with obesity and alteration of glycemic homeostasis
Yeh et al[77]	84 teenagers of Asian origin Country: USA	Genotype frequency (%) TT + CT 63%; CC 37% No difference in BMI, the presence of the T allele associated with increased consumption of carbohydrates and fast food
Yobregon et al[78]	258 Pediatric patients aged 8 to 14 years 115 obese, 42 overweight, 101 eutrophic Country: Chile	Genotype frequency (%) TT-9.7; CT- 38.4; CC- 51.9 No difference in BMI, T allele associated with greater pleasure in eating in obese boys.
Cartel et al[79]	286 Pediatric patients aged 7 to 12 years Country: USA	Genotype frequency (%) TT– 12.9; CT– 39.5; CC- 47.6 TT homozygotes reported 20% higher energy intake of carbohydrates, and greater deposition of visceral fat.

Currently, *FTO* is the gene that shows the most significant association with human polygenic obesity. After evaluating 3337 obese and 3159 controls, a recent meta-analysis study definitively confirms its correlation with obesity in European populations.[27] The association of *FTO* with obesity has been confirmed in other ethnicities[28] such as Asians[29,30] and Hispanics.[31-33] Few studies have evaluated this SNP in the Brazilian population, and only 7 have been carried out in the pediatric population of our country. Chart 2 shows the main findings of these studies.

Recently Sun et al[34] reviewed evidence that suggest a role of two common gene variants, *FTO* and *ANKK1*, in driving gene-environment interactions leading to obesity, metabolic dysfunc-

Chart 2. *Summary of Studies Conducted with Brazilian Children and Adolescents to Evaluate the rs9939609 Polymorphism of the FTO gene*

Reference	Population	Conclusions
Silva et al[59]	N = 348 Age: 0-8-years State: RS	Genotype and allele frequency (%) AA- 16.4;AT- 46.3;TT- 37.4 A - 40;T- 60 Association with higher BMI and adiposity
Lourenco et al[60]	N = 1088 Age: 0-10-years State: AM	Genotype frequency (%) AA- 15.6;AT- 44.8;TT- 39.6 Association with weight gain
Pereira et al[64]	195 obese 153 eutrophic State: MG	Allele frequency (%) Obese: A- 49.5;T-50.5 Eutrophic: A- 46.7;T-53.27 There was no difference between groups
Reuter et al[61]	N = 406 Age: 7-17-years State: RS	Genotype and allele frequency (%) AA-13.3;AT-44.3;TT- 42.4 A - 35.5;T- 64.5 AA genotype associated with obesity/overweight
Nascimento et al[62]	136 obese 172 eutrophic Age: 8-17-years State: PR	Genotype and allele frequency (%) Obese: AA- 14.7;AT- 46.3;TT- 38.9 Eutrophic: AA-9.9; AT-52.3;TT- 37.8 Allele A associated with lower AC reduction after intervention with diet and exercise
Rodrigues et al[65]	378 obesos 378 eutrophic Age:18-9-years State: MA	Genotype and allele frequency (%) Obese: AA -14.5;AT-44.9,TT-40.5 / A-36.8;T-63.2 Eutrophic: AA-13.5; AT- 47.9;TT-38.6 / A- 37.4; T-62.6 There was no difference between groups
Todendi et al[63]	N = 871 Age: 7-17-years State: RS	Allele A associated with increase in BMI

Legend: AC: Abdominal circumference; AM: Amazonas; BMI: Body mass index; MA: Maranhão; MG: Minas Gerais; PR: Paraná; RS: Rio Grande do Sul.

tion, and cognitive change *via* their influence on *DRD2* signaling. It was also demonstrated that the *FTO* protein regulates dopamine signaling *via DRD2*, and the polymorphisms *rs9939609* of the *FTO* gene and *rs18000497* of the *ANKK1* gene interact.[35] A study conducted in humans found that *FTO* risk variants affect the brain dopaminergic response and modify a pleasurable task's learning. The *FTO* gene modulates the BRS connectivity, suggesting that the increased risk for obesity is related to the processing of the sensation of pleasure.[35]

OBJECTIVES

The main objective of this study is to investigate the relationship between pediatric obesity and the polymorphisms of the *ANKK1* (*rs18000497*), *DRD2* (*rs6277*) and *FTO* (*rs9939609*) genes, in a population of Brazilians. We also intend to correlate the studied polymorphisms with the degree of insulin sensitivity and cardiovascular risk factors (CRF) components of the metabolic syndrome (MS): Blood pressure (BP), Abdominal circumference (AC), Triglycerides (TG), High-density cholesterol (HDL) and blood glucose.

METHODS

This research is a Case-Control study carried out jointly by LaGene - Laboratory of Human Cytogenetics and Molecular Genetics of the Health Department of the State of Goiás (LaGene/Lacen/SES-GO), NPR- Replicon Research Center (PUC-GO), and the Children's Hospital of Goiânia/Child Endocrinology Office and Federal University of Goiás (LabMut/FG). The project was approved by the Research Ethics Committee of PUC Goiás, with the number: 16303313.4.0000.0037. All parents or guardians were interviewed and signed the informed consent form consenting with their child's participation in the study and data for research. The researchers clarified the methodological research procedures before and during the study to all participants.

The study included pediatric patients from 5 to16-years of age who sought the pediatric endocrinology office of the Children's Hospital of Goiânia (Goiânia-Goiás-Brazil) years 2017 and 2018. The responsible researcher was personally assessed in complete pediatric consultation with thorough anamnesis and physical examination. Exclusion criteria were: overweight, malnutrition, severe chronic diseases, presence of genetic syndromes, use of medications known to alter weight.

The nutritional status diagnosis was based on the BMI with interpretation according to the reference values proposed by WHO.[36] According to the BMI, children were divided into two main groups: Obese (whose BMI was above +2 SD) and Eutrophic (whose BMI was between -2 SD and +1 SD) that constituted the control group.

The BP was evaluated according to the Hypertension Guidelines for children and Adolescents,[37] which follows the values of the Consensus of the American Academy of Pediatrics.[38] Arterial hypertension was considered if the systolic and/or dia-

stolic blood pressure values were equal to or higher than the 95th percentile for sex, age, and height percentile on three or more occasions.[37,38]

The AC was measured at the midpoint between the last fixed rib (10th) and the iliac crest's upper border. P90 was considered for age, sex, and ethnicity as the maximum value of normality, according to the values described by Freedman and collaborators.[39]

Biochemical Evaluation

After 8 to 12-hours of fasting, blood was collected to determine the lipid profile, blood glucose, and insulin. Through the equipment Architect c8000® Plasma total cholesterol, HDL-cholesterol, and TG were determined by enzymatic-colorimetric assay, and Glucose values were determined by the enzymatic method (Glucose Oxidase - Latest, SP, Brazil). Insulin was measured by the chemiluminescence technique using an immunometric immunoassay in a piece of automated equipment Architect i2000®.

The Brazilian Society of Pediatrics' recommendation for the Brazilian pediatric population was used as the reference for serum lipids.[40] The reference values for fasting blood glucose do not differ between children and adults, with typical values between 60 and 100 mg/dL, pre-diabetes between 101 and 125 mg/dL, and diabetes ≥ 126 mg/dL.[41] Fasting insulinemia is considered normal from 2.5 to 25 mIU/mL in adults and up to 15 mIU/mL in children.[42] The determination of fasting blood glucose and insulin in the same sample allows the calculation of the homeostatic model assessment insulin resistance (HOMA-IR) index, a method used to quantify insulin resistance (IR).[43] The interpretation of HOMA-IR values followed the parameters established by de Almeida et al[44] for the Brazilian pediatric population. HOMA was considered altered when the value was 2 standard deviations (SD) above the mean for age and sex.

According to the value of HOMA-IR, the main groups were subdivided into obese with insulin resistance (ORI) or insulin sensitivity (OSI) and eutrophic resistant (ERI) or sensitive (ESI) to insulin. According to the presence of two or more CRF, they were subdivided into metabolically unhealthy (MU) or metabolically healthy (MH) groups: OMU, OMH, EMU, EMH.

We choose to use the individualized evaluation of CRF and not the presence or absence of MS as there are no reference criteria for MS before ten-years of age, and even after that age, the criteria for MS remain controversial. More than 40 different cut-off points have already been used,[45] and even after the consensus attempt by the International Diabetes Federation, there is still no consensus on which criteria should be used for the diagnosis of MS in adolescence.[46] Thus, this research followed the American Academy of Pediatrics's recommendation to focus on the CRF individually instead of focusing on the diagnosis of MS.[47]

Determination of the Genetic Polymorphisms

The polymorphisms of *DRD2* and *FTO* genes were determined by the real-time polymerase chain reaction (PCR) method. The *rs9939609* and *rs6277* SNPs were genotyped using the TaqMan Real-Time PCR® kit (SNP Genotyping kit, from AppliedBiosystems, USA), following the manufacturer's concentration guidelines. This protocol included the primer oligonucleotide sequences and two fluorophore-labeled TaqMan® minor groove-binding (MGB) probes, named VIC® and FAM™.

To evaluate the polymorphism of the *ANKK1* gene, the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) strategy was used, using the restriction enzyme TaqIA whose cutting site contains an SNP C/T (*C32806T*), following the methodology proposed by Behravan et al.[48]

The Primers used were: GGTTCCTTGCGACTGCTGTGAATTT [T/A] GTGATGCACTTGGATAGTCTCTGTT (Sequence 5´→3´) for FTO; TCTTCTCTGGTTTGGCGGGGCTGTC [G/A] GGAGTGCTGTGGAGACCATGGTGGG (Sequence 5´→3´) for DRD2, and ACCCTTCCTGAGTGTCATCA (Forward) and ACGGCTGGCCAAGTTGTCTA (reverse) for *ANKK1*.

RESULTS

Two hundred twenty-six children and adolescents were evaluated, 124 (54.9%) had obesity, and 102 (45.1%) were eutrophic. The two groups were similar in age and sex distribution (49.2% were female in the obese group and 50.9% in the eutrophic group). Table 1 shows the clinical and biochemical parameters and the proportion of obese and eutrophic patients with altered parameters used for the main groups' subdivision.

All patients were genotyped for the *ANKK1* gene; however, due to an insufficient amount of DNA, two eutrophic and six children with obesity were not genotyped for the *FTO* and *DRD2* genes. For the combined analyzes of the three genes, these eight patients were excluded.

There was no statistical difference between the obese and eutrophic groups in the allelic and genotypic distribution of the three genes studied (Table 2). The risk allele of the three genes was more frequent in the obese group concerning the eutrophic group, and the homozygous genotypes for the wild allele of the *FTO* and *ANKK1* genes were more frequent in the eutrophic group, but none of these differences reached statistical significance.

Table 3 and Figure 2 show the evolution of the mean Z Score of the BMI (Z-BMI) within the obese group, according to the number of risk alleles. In the combined evaluation of the three genes, the presence of 0, 1, and 6 risk alleles was disregarded due to the small number of patients. The highest number of risk alleles correlated with a higher Z-BMI for the *FTO* and *ANKK1* genes individually and in the sum of the three genes studied, showing an upward curve of the lowest number of alleles and a lower Z-BMI for the highest number of alleles and higher Z-BMI (Figure 2), with a statistically significant difference for all ranges of the number of risk alleles considered.

Table 1. *Clinical and Biochemical Parameters Observed in the Obese and Eutrophic Groups*

Variables	Obese		Eutrophic		*p* value
	Mean	SD	Mean	SD	
Age (years)	9.7	2.4	10.1	2.6	0.114
Weight (kg)	55.6	19.8	30.4	10.1	< 0.0001*
Height (cm)	142.7	13.6	135.3	15.7	0.121
Z-BMI	3.05	0.97	- 0.45	0.81	< 0.0001*
Mother´s BMI (kg/m²)	29.7	6.3	24,2	3.3	< 0.0001*
Father´s BMI (kg/m²)	32.4	6.9	26.9	4.7	0.003*
Glucose (mg/dL)	87.08	6.4	86.4	6.7	0.337
Insulin (µui/mL)	12.68	6.7	6.28	2.7	< 0.0001*
HOMA	2.77	1.53	1.32	0.6	< 0.0001*
TC (mg/dL)	170.34	29.9	162.05	26.5	0.13
HDL (mg/dL)	43.25	8.67	48.23	10.01	0.1
LDL (mg/dL)	107.19	29.02	97.37	24.08	0.186
TG (mg/dL)	94.18	44.57	73.65	27.12	< 0.0001*
Variables	**Obese N (%)**		**Eutrophic N (%)**		***p* value**
	Altered	**Normal**	**Altered**	**Normal**	
TG	64 (51.6)	60 (48.4)	31 (30.4)	71 (69.6)	0.0012*
HDL	83 (66.9)	41 (33.1)	40 (39.2)	62 (60.8)	0,0003*
LDL	50 (40.3)	74 (59.7)	25 (24.5)	77 (75.5)	0.0119*
Glucose	2 (1.6)	122 (98.4)	1 (0.9)	101 (99.1)	0.679
BP	4 (3.2)	120 (96.8)	0 (0)	102 (100)	NA
AC	107 (86.3)	17 (13.7)	0 (0)	102 (100)	NA
MS#	83 (66.9)	41 (33.1)	24 (23.5)	78 (76.5)	<0.0001*
Subgroup	OMU	OMH	EMU	EMH	
HOMA	78 (62.9)	46 (37.1)	13 (12.7)	89 (87.3)	<0.0001*
Subgroup	ORI	OSI	ERI	ESI	
Total: N (%)	124 (100%)		102 (100%)		

*Legend: AC: Abdominal circumference; BMI: Body mass index; EIS: Eutrophic with insulin sensitivity; ERI: Eutrophic resistant to insulin resistance, EMH: Eutrophic metabolically healthy; EMU: Eutrophic metabolically unhealthy; HDL: High density cholesterol; LDL: Low density cholesterol; HOMA: Homeostatic Model Assessment Insulin Resistance; ORI: Obese resistant to insulin; OSI: Obese sensitive to insulin; OMH: Obese metabolically healthy; OMU: Obese metabolically unhealthy; BP: Blood pressure; MS: Metabolic syndrome; TC: Total cholesterol; TG: Triglycerides; ; SD: Standard deviation; Z-BMI: Z score of the BMI; # considering 2 or more parameters; *p level with statistical significance.*

Subgroups According to Insulin Sensitivity Criteria

Table 4 shows the genotypic and allelic distribution of the *FTO*, *DRD2* and *ANKK1* genes in the subgroups that were divided according to insulin sensitivity criteria: ORI, OSI, ERI, and ESI.

Regarding the *FTO* gene, in the eutrophic subgroups, the wild homozygous genotype (TT) was more prevalent in the insulin-sensitive group-ESI than in the insulin-resistant group-ERI (36.8×23%, p=0.039). The risk allele (A) was more prevalent in the ERI group (57.7%) than in the ESI group (37.4%), with an odds ratio (OR) of 2.29 for IR when the A allele was present (p=0.048).

Regarding the *DRD2* gene, we did not observe any statistically significant difference in the evaluation of the groups together or separately.

Regarding the *ANKK1* gene: the ERI group showed a higher proportion of the homozygous genotype for the risk allele (TT) when compared to ESI (15.4%×13.5%, p<0.00001).

Subgroups According to the Metabolic Health Criteria

The evaluation of the genotypic and allelic distribution of the *FTO*, *DRD2* and *ANKK1* genes in the groups subdivided according to the CRF number is shown in Table 5.

Regarding the *FTO* gene, in the evaluation of the obese subgroups, we observed that the wild homozygous (TT) genotype is present in 37.5% of the metabolically healthy group (OMH) and 29.5% of the metabolically sick group (OMU) (p=0.02).

Regarding the *DRD2* gene, in the evaluation of the obese subgroups, the homozygous genotype for the non-risk allele (AA) is more prevalent in the healthy subgroup – OMH than in the subgroup with two or more CRF-OMU (32.5%×15.4%, p=0.053). In

Table 2. *Genotypic and Allelic Distribution of FTO, DRD2 and ANKK1 genes in the Obese and Eutrophic Groups. (Risk alleles: FTO=A, DRD2=G, ANKK1=T)*

Gene	Obese N (%)	Eutrophic N (%)	X ²	*p* value
***FTO* rs9939609**				
Genotypes				
AA	25 (21.2)	15 (15)	1.38	0.500
AT	55 (46.6)	50 (50)		
TT	38 (32.2)	35 (35)		
Total	118 (100)	100 (100)		
Alleles				
A	105 (44.5)	80 (40)	0.89	0.344
T	131 (55.5)	120 (60)		
Total	236 (100)	200 (100)		
***DRD2* rs6277**				
Genotypes				
GG	43 (36.4)	29 (29)	1.594	0.450
AG	50 (42.4)	50 (50)		
AA	25 (21.2)	21 (21)		
Total	118 (100)	100 (100)		
Alleles				
G	136 (57.6)	108 (54)	0.577	0.4471
A	100 (42.4)	92 (46)		
Total	236 (100)	200 (100)		
***ANKK1* rs1800497**				
Genotypes				
TT	13 (10.5)	14 (13.7)	1.763	0.414
CT	45 (36.3)	29 (28.5)		
CC	66 (53.2)	59 (57.8)		
Total	124 (100)	102 (100)		
Alleles				
T	71 (28.6)	57 (28)	0.026	0.871
C	177 (71.4)	147 (72)		
Total	248 (100)	204 (100)		

Legend: A: Adenine; C: Cytosine; G: Guanine; T: Thymine.

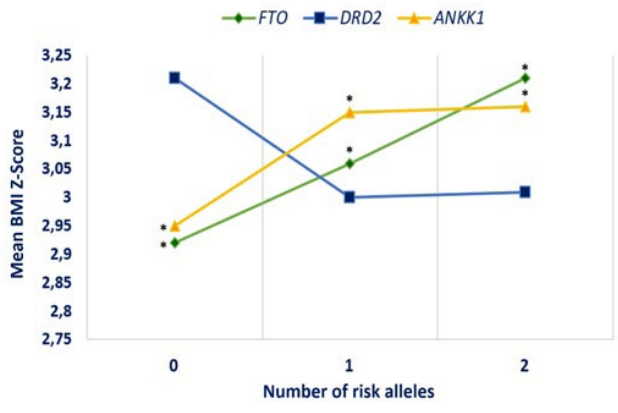
Table 3. *Mean BMI Z score in the Obese Group, According to the Number of Risk Alleles of the FTO, DRD2 and ANKK1 Genes Individually and of the 3 Genes Combined*

Number of Risk Alleles	Mean Z-BMI	SD	*p* value
***FTO* rs9939609**			
0 (TT)	2.92	0.68	0.000*
1 (AT)	3.06	1.09	0.000*
2 (AA)	3.21	1.05	0.000*
***DRD2* rs6277**			
0 (AA)	3.21	1.12	0.453
1 (AG)	3.00	1.05	0.574
2 (GG)	3.01	0.76	0.404
***ANKK1* rs1800497**			
0 (CC)	2.95	0.84	0.000*
1 (CT)	3.15	1.17	0.000*
2 (TT)	3.16	0.76	0.000*
FTO+DRD2+ANKK1			
2	2.89	0.93	0.000*
3	3.09	1.08	0.000*
4	3.13	1.09	0.000*
5	3.45	0.64	0.000*

*Legend: A: Adenine; C: Cytosine; G: Guanine; SD: Standard deviation; T: Thymine; Z-BMI: Z score of the BMI; *p level with statistical significance.*

Figure 2. *Mean BMI Z-Score in the Obese group According to the Number of Risk Alleles of the FTO, DRD2 and ANKK1 Genes*

A) FTO, DRD2 and ANKK1 genes Separately



B) FTO, DRD2 and ANKK1 genes Combined

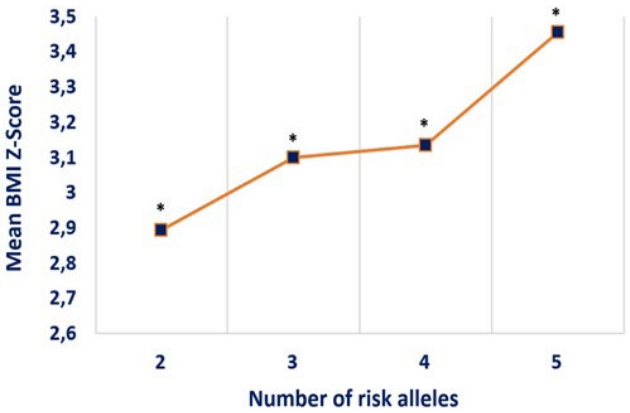


Table 4. *Genotypic and Allelic Distribution of FTO, DRD2 and ANKK1 genes in the Groups: ORI (Obese Resistant to Insulin), OSI (Obese Sensitive to Insulin), ERI (Eutrophic Resistant to Insulin Resistance) and ESI (Eutrophic Sensitive to Insulin)*

Gene	ORI N (%)	OSI N(%)	ERI N(%)	ESI N(%)	X ²	*p* value
***FTO* rs9939609**						
Genotypes						
AA	15 (20)	10 (23.3)	5 (38.5)	10 (11.5)		
AT	36 (48)	19 (44.2)	5 (38.5)	45 (51.7)	7.15	a0.039
TT	24 (32)	14 (32.5)	3 (23)	32 (36.8)		
AA+AT	51 (68)	29 (67.5)	10 (77)	55 (63.2)	1.14	0.765
TT	24 (32)	14 (32.5)	3 (23)	32 (36.8)		
Total	75 (100)	43 (100)	13 (100)	87 (100)		
Alleles						
A	66 (44)	39 (45.3)	15 (57.7)	65 (37.4)		
T	84 (56)	47 (54.7)	11 (42.3)	109 (62.6)	4.76	a0.048
Total	150 (100)	86 (100)	26 (100)	174 (100)		
***DRD2* rs6277**						
Genotypes						
GG	27 (36)	16 (37.2)	5 (38.5)	24 (27.6)		
AG	28 (37.3)	22 (51.2)	5 (38.5)	45 (51.7)	6.54	0.3649
AA	20 (26.7)	5 (11.6)	3 (23)	18 (20.7)		
GG+AG	55 (73.3)	38 (88.4)	10 (77)	69 (79.3)	3.75	0.289
AA	20 (26.7)	5 (11.6)	3 (23)	18 (20.7)		
Total	75 (100)	43 (100)	13 (100)	87 (100)		
Alleles						
G	82 (54.7)	54 (62.8)	15 (57.7)	93 (53.4)		
A	68 (45.3)	32 (37.2)	11 (42.3)	81 (46.6)	2.20	0.530
Total	150 (100)	86 (100)	26 (100)	174 (100)		
***ANKK1* rs1800497**						
Genotypes						
TT	9 (11.5)	4 (8.7)	2 (15.4)	12 (13.5)		
CT	28 (35.9)	17 (37)	3 (23.1)	26 (29.2)	2.18	0.414
CC	41 (52.5)	25 (54.3)	8 (61.5)	51 (57.3)		
TT+CT	37 (47.5)	21 (45.7)	5 (38.5)	38 (42.7)	0.60	0.895
CC	41 (52.5)	25 (54.3)	8 (61.5)	51 (57.3)		
Total	78 (100)	46 (100)	13 (100)	89 (100)		
Alleles						
T	46 (29,5)	25 (27,2)	7 (26,9)	50 (28,1)		
C	110 (70,5)	67 (72,8)	19 (73,1)	128 (71,9)	0.19	0.978
Total	156 (100)	92 (100)	26 (100)	178 (100)		

Legend: A: Adenine; C: Cytosine; EIS: Eutrophic with insulin sensitivity; ERI: Eutrophic resistant to insulin; G: Guanine; ORI: Obese resistant to insulin; OSI: Obese sensitive to insulin; T: Thymine; a – p value in the comparison between ERI and ESI.

Table 5. *Genotypic and Allelic Distribution of the FTO, DRD2 and ANKK1 Genes in the Groups: OMU (Obese MetaBolically Unhealthy), OMH (Obese Metabolically Healthy), EMU (Eutrophic Metabolically Unhealthy) and EMH (Eutrophic Metabolically Healthy)*

Gene	OMU N (%)	OMH N(%)	EMU N(%)	EMH N(%)	X ²	*p* value
***FTO* rs9939609**						
Genotypes						
AA	12 (15.4)	13 (32.5)	4 (17.4)	11 (14.3)		
AT	43 (55.1)	12 (30)	12 (52.2)	38 (49.3)	9.88	a0.020
TT	23 (29.5)	15 (37.5)	7 (30.4)	28 (36.4)		
AA + AT	55 (70.5)	25 (62.5)	16 (69.6)	49 (63.6)	1.23	0.745
TT	23 (29.5)	15 (37.5)	7 (30.4)	28 (36.4)		
Total	78 (100)	40 (100)	23 (100)	77 (100)		
Alleles						
A	67 (42.9)	38 (47.5)	20 (43.5)	67 (42.9)		
T	89 (57.1)	42 (52.5)	26 (56.5)	89 (57.1)	1.63	0.650
Total	156 (100)	80 (100)	46 (100)	154 (100)		
***DRD2* rs6277**						
Genotypes						
GG	28 (35.9)	15 (37.5)	7 (30.4)	22 (28.6)		
AG	38 (48.7)	12 (30)	12 (52.2)	38 (49.4)	7.53	a0.053
AA	12 (15.4)	13 (32.5)	4 (17.4)	17 (22)		
GG + AG	66 (84.6)	27 (67.5)	19 (82.6)	60 (88)	4.88	a0.031
AA	12 (15.4)	13 (32.5)	4 (17.4)	17 (22)		
Total	78 (100)	40 (100)	23 (100)	77 (100)		
Alleles						
G	94 (60.3)	42 (52.5)	26 (56.5)	82 (53.2)		
A	62 (39.7)	38 (47.5)	20 (43.5)	72 (46.8)	2.02	0.567
Total	156 (100)	80 (100)	46 (100)	154 (100)		
***ANKK1* rs1800497**						
Genotypes						
TT	49 (59)	4 (9.8)	5 (20.8)	44 (56.4)		
CT	25 (30.1)	20 (48.8)	4 (16.7)	25 (32.1)	9.01	b0.031
CC	9 (10.9)	17 (41.4)	15 (62.5)	9 (11.5)		
TT + CT	34 (41)	24 (58.5)	9 (37.5)	34 (53.6)	4.18	0.242
CC	49 (59)	17 (41.5)	15 (62.5)	44 (46.4)		
Total	83 (100)	41 (100)	24 (100)	78 (100)		
Alleles						
T	43 (25.9)	28 (34.1)	14 (29.2)	43 (27.6)		
C	123 (74.1)	54 (65.9)	34 (70.8)	113 (72.4)	0.9	0.591
Total	166 (100)	82 (100)	48 (100)	156 (100)		

Legend: A: Adenine; C: Cytosine; EMH: Eutrophic metabolically healthy; EMU: Eutrophic metabolically unhealthy; G: Guanine; OMH: Obese metabolically healthy; OMU: Obese metabolically unhealthy; T: Thymine; a – p value in the comparison between OMU and OMH, b – p value in the comparison between OMH e EMU.

patients with obesity, the genotypes with the presence of the risk allele of the *DRD2* gene (GG+AG) increased the risk for metabolic disease by 2.65 times (p=0.031).

Regarding the *ANKK1* gene, when we compared the "healthy obese" group with the "unhealthy eutrophic" group, we observed that the homozygous genotype for the risk allele (TT) was twice as frequent in the EMU group as in the OMH group (20.8%×9.8%, p=0.031).

DISCUSSION

The polygenic nature of common obesity discovers risky genes and their variants a challenging task. Genome-Wide Association Study (GWAS) studies have brought new insights into the understanding of polygenic obesity, but even today, specific genes' contribution

to BMI variability is still poorly understood.[6] No GWAS study has included the Brazilian population, and studies in the pediatric age group are scarce. Although carried out with a small number of patients and with groups composed following the simple random sampling strategy without replacement, the present research contributed to the knowledge of genetic factors that interfere in the phenotype of pediatric obesity in Brazilians.

The presence of higher-levels of parent's BMI of the obese children corroborates studies that indicate a vital genetic component in human obesity.[49-51] In addition to the genetic factor, these children live in the same environment as their family, therefore, they are exposed to their lifestyle. It is known that children whose both parents are obese have an 80% possibility of being obese, 50% when one parent is obese, and 9% when the parents are not obese.[52] It is worth mentioning that the father's average BMI is above normal in both groups. The group of eutrophic children has overweight fathers, a fact consistent with the advancement of overweight and obesity in Brazilians, which affects 56.9% of the adult population.[53]

Both obese and eutrophic groups had similar blood glucose levels. The obese group, however, showed higher-levels of insulin and HOMA-IR. These insulin sensitivity changes are attributed to obesity per se, which is a significant risk factor for diabetes mellitus (DM) type 2. The worsening of the situation of the pancreatic malfunction is related to obesity evolves to DM.[54-56]

We observed that TG levels were higher in the obese group, an expected result since these children also had elevated insulin and HOMA. Hypertriglyceridemia is a pervasive alteration in patients with obesity and is related to insulin resistance: obese patients with IR, even without DM2 installed, have selective hepatic IR: insulin cannot suppress hepatic glucose production while continuing to perpetuate lipogenesis, resulting in hypertriglyceridemia.[57,58]

The other lipid profile parameters, the mean TC, high-density cholesterol (HDL), and low-density cholesterol (LDL), did not show a statistical difference. The mean levels of TC and LDL were higher in the obese group but still within the normal range, while the mean value of HDL was lower in the obese group and was in the sub-normal range. When assessing the number of individuals in each group with LDL and HDL outside the normal range (Table 1), a significantly higher proportion of obese people was in this condition. Indeed, a similar means between the obese and eutrophic groups might be due to the presence of 33.1% of "Healthy Obese" (OMH group) and 23.5% of "Sick Eutrophic," also called "false thin" or "obese eutrophic," that composed the EMU group.

It was observed that the risk allele of the three genes was more frequent in the obese group and that the homozygous genotype for the non-risk allele of the *FTO* and *ANKK1* genes was more frequent in the eutrophic, but none of these differences reached statistical significance. The small number of participants evaluated may justify the lack of statistical significance.

When evaluating the effect of the presence of the risk alleles within the obese group, we observed that the higher the number of risk alleles, the greater the Z-BMI for the *FTO* and *ANKK1* genes alone, and in the sum of the 3 genes studied. The upward curve of the lowest number of alleles and lowest Z-BMI for the highest number of alleles and the highest Z-BMI, with a statistically significant difference for all ranges of the number of risk alleles considered, indicate that the studied polymorphisms have an additive effect on the outcome of childhood-onset obesity. This result is unprecedented in the literature.

Considerations Regarding the FTO gene rs9939609

The *rs9930506* polymorphism of the FTO gene is the SNP that shows the most significant association with human polygenic obesity: a recent meta-analysis study after evaluating 3337 obese and 3159 controls confirms its correlation with obesity in European populations.[27] Few studies have evaluated this SNP in the Brazilian population, and only seven were carried out in the pediatric population of our country, five have found an association with adiposity[59-63] and 2 showed no difference between patients with or without obesity.[64,65]

Our study showed that in the Eutrophic group, the presence of wild homozygous genotype (TT) associates with insulin sensitivity, and the presence of the risk allele (A) associates with an increase of 2, 29 times in the risk for IR. This SNP has already been associated with insulin sensitivity changes in insulin sensitivity.[66] but this is the first time this result is presented in Brazilian children. Within the obese group, the TT genotype was more frequent in the OMH group (37.5%) than in the OMU group (29.5%); this difference is statistically significant, so we can say that the T allele is associated with the absence of CRF, which can be considered a protective factor within the obese group, a result also unprecedented in the literature for the Brazilian population.

Considerations Regarding the *DRD2* gene rs6277

The *rs6277* is a poorly studied SNP: only six scientific studies have evaluated the rs6277 variant of the *DRD2* gene in children or adolescents to assess cognitive control and behavioral nuances[67-71] and choke risk.[72]

From the metabolic point of view, the *rs6277* polymorphism of the *DRD2* gene was evaluated in only four articles, all performed in adults. This SNP was associated with compulsive eating disorder,[17,18] weight gain,[19] and higher sugar consumption.73,74 The prevalence of the GG genotype varied between 12 and 21% in these studies. Our series found a higher frequency of genotypes with the risk allele (G), GG-36.4 and 29%, AG-42.4%, and 50% in obese and eutrophic individuals respectively, and AA - 21% for both groups. The highest frequency of the G allele found in our study is probably due to the great miscegenation of the Brazilian population, data corroborated by the 1000 Genome Project,75 which shows a frequency of the G allele of 12.5% in Europeans, 8% in Asians, and 93% in Africans.

Ramos-Lopez et al[73] associated the GG genotype with

significantly higher-levels of TG in adults. The present study associated the G allele of the SNP rs 6277 of the *DRD2* gene with CRF in obese children and adolescents, which was an unprecedented result in the literature.

Considerations Regarding the *ANKK1* gene rs1800497

We observed that the frequency of the homozygous genotype for the wild allele (CC) was slightly higher in the eutrophic group (57.8%) than in the obese group (53.2%). However, there was no statistically significant difference in the allelic or genotypic distribution. This result disagrees with a previous study carried out by our team that found an association of the T-allele with the presence of obesity in children.[74,75] However, when assessing the evolution of the mean Z-BMI in the obese group, we observed that the higher the number of risk alleles, the higher the Z-BMI, corroborating the previous finding that the T allele is associated with increased BMI.

Few studies have been carried out to verify the association of the *rs1800497* polymorphism of the *ANKK1* gene in children and adolescents. A considerable variation in allele frequencies was observed in the literature, even within populations of the same country, and inconsistency regarding the association with metabolic outcomes.[12,76-79]

In evaluating the subgroups using the HOMA values criterion, the risk allele's homozygous genotype (TT) was associated with insulin resistance in eutrophic individuals. This result corroborates the finding of previous research carried out by our group, which for the first time in the literature, associated the T-allele of the *ANKK1* gene with alteration of glycemic homeostasis.[76]

Clinical and animal studies evidence the relationship between *DRD2* receptors and glucose metabolism. Clinical research conducted in diabetic patients[80] and animal study[81] demonstrated improved glycemic control using Bromocriptine, a dopaminergic agonist that acts *via DRD2* receptors. In 2005, RUBI et al[82] demonstrated for the first time that *DRD2* receptors are expressed in the pancreatic beta-cell and modulate insulin secretion. A study carried out in knockout rats for the *DRD2* gene revealed that *DRD2* receptors play a crucial role in insulin secretion and glycemic homeostasis; rats with no *DRD2* receptor showed a failure in insulin response in the face of glucose overload, higher fasting glucose, glucose intolerance, and reduced beta-cell mass.[83] Such results show that *DRD2* is essential for the proliferation of beta cells and insulin secretion and can be considered a growth factor essential for glycemic homeostasis.[83]

Regarding the presence of CRF, the risk allele of the *ANKK1* gene was associated with the presence of two or more CRF, as shown by the presence of the homozygous risk genotype (TT) in 20.8% of EMU and only 9.8% of OMH, a result also found to be unprecedented in the literature.

CONCLUSION

In conclusion, our results found that the risk alleles of the *FTO*, *DRD2* and *ANKK1* genes interfered with the outcome of pediatric obesity in Brazilian children:

- The higher number of the risk alleles of *FTO* and *ANKK1* genes and the 3 genes combined significantly increased the mean Z-BMI of the obese group.
- The risk alleles of the *FTO* and *ANKK1* genes were positively associated with IR in eutrophic children.
- The T allele of the *FTO* gene has a protective effect against CRF in children with obesity
- The risk alleles of the *DRD2* and *ANKK1* genes conferred a higher risk of CRF in children with obesity and normal weight, respectively.

For being a case-control, our findings cannot ascribe causality, only association. Further studies are needed, with a more significant number of patients and longitudinal follow-up, to confirm the possible causality between the risk alleles and our findings.

It is hoped that in the future, more excellent knowledge of the specific contribution of each genetic variant will be a useful tool in clinical practice, being able to guide preventive measures and specific treatment according to genetic risk scores.

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- RMP and ADC designed the research (project conception, development of overall research plan, and study oversight).
- RMP conducted the clinical evaluation JSF, RVM, and LBM conducted the genetic protocols (hands-on conduct of the experiments).
- RMP, LBM, and ADC analyzed data or performed statistical analysis.
- RMP, MPC, and ADC interpreted data findings.
- RMP, JSF, MPC, and ADC wrote the paper.
- RMP and ADC had primary responsibility for final content. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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